2,5-Dialkyltetrahydrofurans, Common Components of the Cuticular Lipids of Lepidoptera

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In more than 50 lepidopteran species 2,5-dialkyltetrahydrofurans were identified as components of the cuticular lipids. The chain length of these compounds varies between C_{25} and C_{37} and both *cis*- and *trans*-compounds are present. In addition, previously unknown 2-alkyl-5-(1-hydroxyalkyl)tetrahydrofurans were found in some species. The identification procedure and synthesis of representative compounds are reported.

Introduction

The cuticle of insects is covered with a lipid layer the primary function of which is to prevent desiccation. Cuticular lipids can consist of hydrocarbons (the major group of compounds in most species), alcohols, aldehydes, ketones, wax esters, and fatty acids. In addition, dialkyl ethers, glyceride ethers or triglycerides have been found (Nelson and Blomquist, 1995).

Recently it has been shown that the parasitoid *Apanteles kariyai* locates its host, the common armyworm *Pseudaletia separata,* by detection of 2,5-dialkyltetrahydrofurans present on the surface of the larvae (Takabayashi and Takahashi, 1986a, b). During our studies on pheromones of danaine butterflies we identified similar compounds associated with the male hairpencils (Schulz *et al.,* 1993; Schulz and Nishida, 1996). In this study we show that 2,5-dialkyltetrahydrofurans occur as constituents of cuticular lipids of various genera of adult Lepidoptera and discuss their identification and the synthesis of new derivatives.

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Material and Methods

Samples, analysis and sample preparation

We studied the cuticular lipids of adult male butterflies of over 50 species, collected from different sources worldwide (Table I). For comparison we also examined males from a limited number of moth species (Table II). Finally, as tetrahydrofurans (THFs) present in cuticle of young *Pseudaletia separata* larvae serve as a kairomone for parasitic Hymenoptera (Takabayashi and Takahashi, 1986a, b), we compared the cuticular profiles of early and late instar larvae, as well as adult males of the true armyworm, *P. unipuncta.* Whole insects or body parts (see Tables I and II) were extracted with pentane or dichloromethane and stored at −40 °C until analysed by gas chromatography – mass spectrometry (GC–MS). Mass spectra (70 eV) were obtained with a VG 70/250 S mass spectrometer coupled to a Hewlett-Packard HP 5890 A gas chromatograph and a Fisons MD-800 mass spectrometer coupled to a Fisons GC 8000. Gas chromatographic analyses were carried out with a Carlo-Erba Fractovap 2101 gas chromatograph equipped with a flame

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ionization detector and on-column or split/splitless-injection. Separations were performed on capillary columns with apolar phases like Rt_x-5, DB-5, CP-Sil-8, or BPX-5. The last mentioned phase was used for the determination of retention indices. ^1H NMR and ^{13}C NMR spectra were obtained with Bruker WM 400 or AC250P instruments. Silylations were performed by adding 50 µl MSTFA (*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide) to 50 µl of a lipidic extract. After 30 minutes at 60 °C, the silylated samples were concentrated under a stream of nitrogen and then analyzed.

Synthesis of representative compounds

For confirmation of our identifications, several THFs were synthesized (see Fig. 1). Furan was consecutively alkylated twice according to the method of Brandsma and Verkruijsse (1986). The 2,5-dialkylfuran obtained was then hydrogenated over palladium/charcoal, to yield a 99:1 mixture of *cis*- and *trans*-2,5-dialkyltetrahydrofurans. The alcohol 2-heptyl-5-(1-hydroxyoctadecyl)-tetrahydrofuran (C7/1HOC18-THF) was synthesized starting from 2-heptylfuran. After lithiation of the furan, octadecanal was added. The resulting 2-heptyl-5-(1-hydroxyoctadecyl)-furan can be hydrogenated with rhodium on charcoal to yield a 1:1 mixture of the two diastereomers of *cis*-C7/1HOC18-THFs, which elute as one peak on an apolar gas chromatographic phase. Attempts to separate the enantiomers on different chiral cyclodextrine phases were unsuccessful. The synthetic compounds exhibited identical mass spectra and retention times to the natural compounds.

2-Heptyl-5-octadecylfuran

2-Heptylfuran was prepared according to the method of Brandsma and Verkruijsse (1986), by alkylation of furan with heptyl bromide. A second alkylation with octadedyl bromide yielded 2-heptyl-5-octadecylfuran.

^1H NMR (250 MHz, $CDCl_3$): δ = 0.95 (t, 6H, CH_3), 1.24–1.40 (m, 38H, CH_2), 1.63 (quin, 4H, H-2′), 2.60 (t, 4H, H-1′, *J* = 7.8 Hz), 5.92 (s, 2H, H-3, H-4).

EI-MS (70 eV): *m/z* (%) = 43 (72), 57 (39), 81 (24), 95 (85), 107 (32), 127 (8), 179 (100), 193 (22), 291 (3), 323 (35), 337 (12), 418 (83, M^+).

2-Heptyl-5-octadecyltetrahydrofuran

A solution of 2-heptyl-5-octadecylfuran (50 mg) in 5 ml hexane was hydrogenated at 0.5 bar with 10% Pd/C as catalyst. After filtration, a 99:1 *cis/trans* mixture of pure 2-heptyl-5-octadecyltetrahydrofuran was obtained.

^1H NMR (250 MHz, $CDCl_3$): δ = 0.88 (t, 6H, CH_3), 1.20–1.45 (m, 42H, CH_2), 1.60 (quin, 4H, H-1′), 1.90 (m, 2H, H-3, H-4), 3.65–3.90 (m, 2H, H-2, H-5).

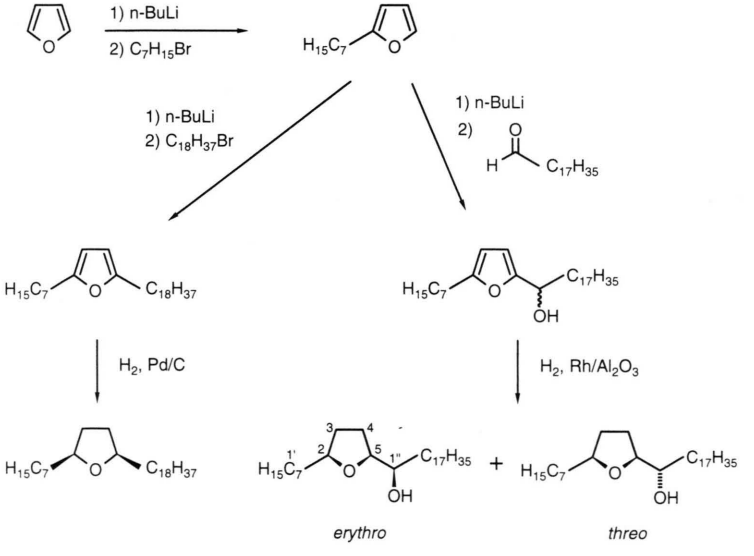

Fig. 1. Synthesis of 7/18-THF and 7/1HO18-THF.

^{13}C NMR (62.9 MHz, C_6D_6): δ = 14.4 (C-7′, C18″), 23.1 (C-6′, C-17″), 26.9 (C-2′), 29.8–30.3, 31.6 (C-3), 32.3 (C-5′), 32.4 (C-16′), 36.8 (C-1), 79.4 (C-2, C-5).

2-Heptyl-5-(1-hydroxyoctadecyl)-furan

A 1.6 M solution of *n*-butyllithium in hexane (1.5 ml) was added to a stirred solution of 2-heptylfuran (390 mg, 2 mmol) in 4 ml THF at 0 °C. After 2 h, a solution of octadecanal (500 mg, 1.87 mmol) in 4 ml THF was added dropwise and the resulting mixture stirred for 3 h at room temperature. A saturated NH_4Cl solution was added, and the mixture extracted three times with diethyl ether. The combined organic extracts were dried with $MgSO_4$ and the solvent removed. The crude extract was purified by chromatography on 100 g neutral Al_2O_3 (Woelm, activity 3–4), giving 430 mg of pure 2-heptyl-5-(1-hydroxyoctadecyl)-furan (62% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 6H, *J* = 6.8 Hz, CH_3), 1.21–1.46 (m, 38H, CH_2), 1.62 (quin, 2H, *J* = 7.4 Hz, CH_2-CH_2-furyl), 1.83 (m, 2H, CH_2-CHOH), 2.59 (t, 2H, *J* = 7.2 Hz, CH_2-furyl), 4.60 (t, 1H, *J* = 6.6 Hz, CH-OH), 5.89 (d, 1H, *J* = 3.0 Hz, CH), 6.10 (d, 1H, *J* = 3.0 Hz, CH).

2-Heptyl-5-(1-hydroxyoctadecyl)-tetrahydrofuran

A solution of 50 mg 2-heptyl-5-(1-hydroxyoctadecyl)-furan in 3 ml of a 3:1 methanol/diethyl ether mixture was stirred together with 10 mg of a 5% Rh/Al_2O_3 catalyst for 3 h under an atmosphere of hydrogen of 0.2 bar. The mixture was filtered and the solvent removed to give almost pure 2-heptyl-5-(1-hydroxyoctadecyl)-tetrahydrofuran. Small amounts of 2-heptyl-5-octadecyltetrahydrofuran, formed by hydrogenolysis of the hydroxy group, were removed by chromatography on Al_2O_3. The analysis of NMR spectra according to Fujimoto *et al.* (1994) showed that a 1:1 mixture of the *threo*- and *erythro-cis*-hydroxyalkyltetrahydrofurans was formed.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 6H, *J* = 6.8 Hz, CH_3), 1.20–2.00 (m, CH_2), 3.36 (q, 1H, *J* = 6.1 Hz, *threo*-CH-OH), 3.70 (q, 1H, *J* = 6.6 Hz, *threo*-CH-O-C), 3.77–3.89 (m, 4H, *threo*- and *erythro*-CH-O-C, *erythro*-CH-OH).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1 (C-7′, C-18″), 22.6 (C-6′, C-17″), 23.8 (*erythro*-C-4), 25.7 (*threo*-C-3″), 26.0 (*erythro*-C-3″), 26.2 (*erythro*-C-2′), 27.8 (*threo*-C-4), 29.2–29.7 (CH_2), 31.4 (C-3), 31.8 (C-5′), 31.9 (C-16″), 32.6 (*erythro*-C-2″), 34.0 (*threo*-C-2″), 35.9 (*erythro*-C-1′), 36.0 (*threo*-C-1′), 71.6 (*erythro*-C-1″), 74.5 (*threo*-C-1″), 79.6 (*erythro*-C-2), 79.9 (*threo*-C-2), 82.0 (*erythro*-C-5), 82.2 (*threo*-C5).

Results

In the current study, cuticular lipids of adult butterflies belonging to the Danainae, Heliconiinae, Ithomiinae (all Nymphalidae) and Pieridae, as well as noctuid, arctiid, pyralid, tortricid, and plutellid moth species were investigated. Because our primary interest is the chemical communication systems of male Lepidoptera, we analyzed body parts associated with suspected pheromone emitting structures. In some cases other body parts or live stages as well as females of the ithomiine *Methona confusa* were investigated, additionally (see Tables I and II).

The lipids were extracted as described and the extracts submitted to GC-MS. Long chain unbranched 2,5-dialkyltetrahydrofurans and related alcohols were identified in addition to the usual alkanes and other compounds. They exhibited distinct mass spectra, permitting easy location of the ring in the chain (Brandt and Djerassi, 1968; Zinbo and Jensen, 1985; Takabayashi and Takahashi, 1986a). The mass spectrum of 2-nonyl-5-octadecyltetrahydrofuran (9/18-THF*) is depicted in Fig. 2A. The α-cleavage next to the ring gives rise to intense $C_nH_{2n-1}O$-ions (A) that indicates the length of each side chain. These fragments are accompanied by characteristic small C_nH_{2n-3}-ions (B), formed by additional loss of water. The molecular weight can be determined by the small ions M-1 and M-18.

In some species hydroxylated tetrahydrofuran derivatives were identified, which showed mass spectra exhibiting only one intense ion A. The spectrum of 2-(1-hydroxyoctadecyl)-5-nonyltetrahydrofuran (C9/1HOC18-THF) is shown in Fig. 2B. The highest visible ion is *m/z* = 448, which arises by loss of water from the molecular ion. The

* The numbers indicate the lengths of the side chains. Thus, 2-octadecyl-5-tridecyltetrahydrofuran is abbreviated as 13/18-THF.

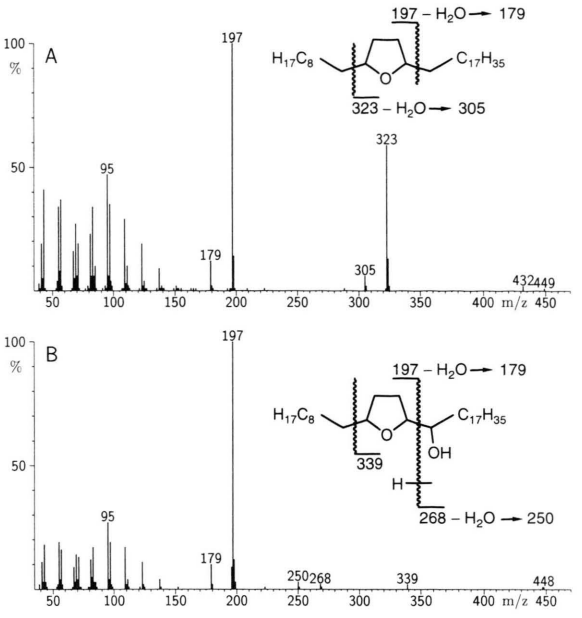

Fig. 2. Mass spectrum and fragmentation pattern of A: 2-octadecyl-5-nonyltetrahydrofuran (C9/C18-THF) B: 2-(1-hydroxyoctadecyl)-5-nonyltetrahydrofuran (C9/1HOC18-THF).

C-9 side chain can be deduced from the base peak at m/z = 197. The second fragment A plus an additional oxygen was observed at m/z = 339. The position of the oxygen atom is probably at C-1 of the octadecyl side chain, because α-cleavage on the THF side plus hydrogen loss leads to m/z = 268, which is accompanied by water elimination to furnish the ion m/z = 250. Silylation was performed to prove that the additional oxygen is part of a hydroxyl group. In the spectrum of silylated C7/1HOC20-THF (Fig. 3) the base peak was found at m/z = 369. This ion is formed by cleavage between the silyloxy group and the tetrahydrofuran ring. The ions A are of low abundance and are found at m/z = 169 and m/z = 439. The identifications were verified by synthesis of reference compounds (see Materials and Methods).

Dialkyltetrahydrofurans with chain lengths between C_{25} and C_{37} were identified in cuticular lipids of 55 Lepidoptera species investigated (see Tables I and II). They occurred in mixtures containing both the *cis*- and *trans*-isomers, but these were not present in identical concentrations. On apolar phases, the *cis*-componds elute earlier than the respective *trans*-components. The retention indices of four synthetic compounds with a C_{29} carbon backbone were determined as follows: *cis*-11/14-THF 2963, *trans*-11/14-THF 2977, *cis*-9/16-THF 2967, *trans*-9/16-THF 2979, *cis*-7/18-THF 2974, *trans*-7/18-THF 2983, *cis*-5/20-THF 2984, and *trans*-5/20-THF 2991. The hydroxylated compound *cis*-7/1HO18-THF has a retention index of 3177.

Tetrahydrofurans with an odd number of carbons in the chain predominate in the natural waxes. The ring is always located in the middle of the chain and contains substituents which vary in length between C_5 and C_{22}. Positional isomers of the major components often show a difference of two carbons in the location of the ring. For example, the cuticular lipids of the ithomiine butterfly *Ithomia salapia* contain major amounts of 11/18-THF and 13/16-THF as well as 13/18-THF and 15/16-THF. The major components usually contain a C_{16}-, C_{18}-, or C_{20} side chain and possess a total of 31, 33, or 35 carbon atoms. They are accompanied by tetrahydrofurans with an even number of carbons that do not show specific positional preferences. The natural mixtures can be quite complex. For example, more than 60 THF's were identified as constituents of the cuticular lipids of *Amauris niavius* after fractionation (Schulz *et al.*, 1993).

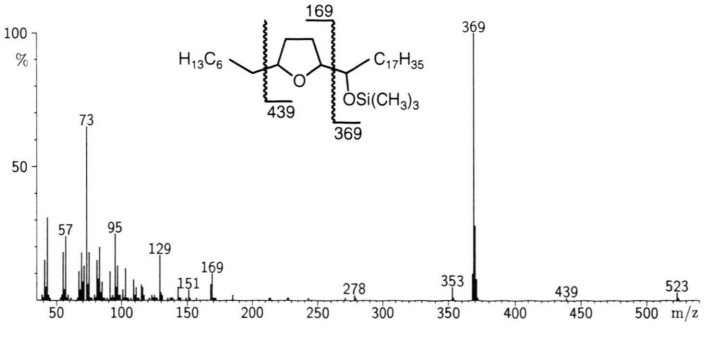

Fig. 3. Mass spectrum and fragmentation pattern of 2-heptyl-5-(1-trimethylsilyloxyoctadecyl)-tetrahydrofuran.

Table I. Species, origin and major dialkyltetrahydrofurans present in the surface of butterflies. Body parts refers to the parts analyzed. WI: wings, WF: wings of female, CL: clasper; HP: hair pencils; BO: whole insect. A following + denotes inclusion of pheromone emitting structures in the sample, a – the opposite.

Species	Origin	Body parts	Major tetrahydrofurans
Pieridae			
Catopsilia pomona	Sri Lanka	WI+, WI–	11/16
Pieris rapae	Germany	BO+	not detected
Nymphalidae			
Danainae			
Amauris albimaculata	Kenya	HP+, WI+	7/18, 9/18
Amauris damocles	Togo	HP+, WI+	7/20, 9/18
Amauris echeria	Kenya	HP+, WI+	7/20, 9/18
Amauris hecate	Kenya	HP+	7/20, 9/18, 9/20
Amauris niavius[1]	Kenya	HP+, WI+	7/20, 9/18, 9/20, 11/18
Amauris ochlea	Kenya	HP+, WI+, WI–	9/20
Amauris tartarea	Kenya	HP+, WI+	9/18
Danaus affinis	Papua New Guinea	HP+, WI+	11/16
Danaus chrysippus	Kenya	HP+, WI+	11/16, 11/18, 13/16
Danaus genutia	Sri Lanka	HP+, WI+, WI–	11/16
Danaus gilippus	USA	HP+, WI+	11/16
Euploea core	Sri Lanka	HP+	11/16
Euploea klugii	Sri Lanka	HP+	11/16, 13/14
Idea leuconoe[2]	Japan	HP+, WI–	9/16, 9/18
Parantica aglea	Sri Lanka	HP+, WI+, WI–	7/18
Tirumala limniace	Sri Lanka	HP+, WI+, WI–	11/16
Ithomiinae			
Callithomia hezia	Costa Rica	WI+	not detected
Ceratinia tutia	Ecuador	WI+	11/18, 13/16
Dircenna dero	Argentina	WI+	13/16, 13/18
Dircenna loreta	Ecuador	WI+	13/16
Godyris zavaleta	Ecuador	WI+	11/18
Heterosais edessa	Brazil	WI+	not detected
Heterosais nephele	Ecuador	WI+	11/18, 13/16
Hypoleria orolina	Ecuador	WI+	9/20, 11/18, 13/16
Hypomenitis andromica	Ecuador	WI+	11/18, 13/16
Hyposcada illinissa	Ecuador	WI+	11/18, 13/16
Hypothyris anastasia	Brazil, Ecuador	WI+, WI–	9/20, 11/20, 13/18
Hypothyris euclea	Ecuador	WI+	11/18, 13/16, 13/18, 15/16
Hypothyris mamercus	Ecuador	WI+	11/20, 13/18, 15/16
Hypothyris moebiusi	Ecuador	WI+	11/18, 13/16, 13/18, 15/16
Ithomia agnosia	Ecuador	WI+	not detected
Ithomia salapia	Ecuador	WI+	11/18, 13/16, 13/18, 15/16
Mechanitis polymnia	Brazil, Ecuador	WI+	13/18, 15/16
Mechanitis lysimnia	Brazil, Ecuador	WI+	13/18, 15/16
Melinaea ludovica	Brazil	WI+, WI–	7/20, 9/20
Melinaea menophilus	Brazil	WI+, WI–	11/18, 13/16
Methona confusa	Ecuador, Brazil	WI+, WI–, WF+	9/16, 9/18, 9/20, 11/18
Napeogenes sylphis	Ecuador	WI+	11/16, 13/18
Oleria gunilla	Ecuador	WI+	13/18, 15/16
Oleria padilla	Ecuador	WI+	9/18, 11/16, 11/18, 13/16
Pseudoscada timna	Ecuador	WI+	11/16, 11/18, 13/18
Pteronymia vestilla	Brazil	WI+	11/18, 13/16
Prittwitzia hymenaea	Argentina	WI+	11/18, 13/16
Scada kusa	Ecuador	WI+	9/18, 11/16, 11/18, 13/16
Thyridia psidii	Brazil	WI+, WI–	7/20, 9/18, 9/20
Tithorea harmonia	Brazil, Ecuador	WI+	9/18, 11/16, 11/18, 13/16
Tithorea tarricina	Costa Rica	WI+	11/16

Table I. Continued.

Species	Origin	Body parts	Major tetrahydrofurans
Heliconiinae			
Argynnis paphia	Germany	CL+, WI+, WI–	11/18
Dryas iulia	Costa Rica	CL+, WI+	not detected
Heliconius charitonia	Costa Rica	CL+	9/18, 11/16
Heliconius cydno	Costa Rica	CL+	11/18, 9/20, 11/20, 13/18
Heliconius doris	Costa Rica	CL+, WI+	9/18, 11/16, 11/18, 13/16
Heliconius erato	Costa Rica	CL+, WI+	not detected
Heliconius hecale	Costa Rica	CL+, WI+	9/16, 9/18, 11/16
Heliconius hewitsoni	Costa Rica	CL+, WI+	not detected
Heliconius ismenius	Costa Rica	CL+, WI+	7/20
Heliconius melpomene	Costa Rica	CL+, WI+	not detected
Heliconius telesiphe	Costa Rica	CL+, WI+	9/16, 11/18, 11/16, 13/18

[1] Schulz *et al.* (1993).
[2] Schulz and Nishida (1996).

Table II. Species, origin and major dialkyltetrahydrofurans present in the surface lipids of adult moths. Body parts refers to the parts analyzed. LE: legs; CL: clasper; BO: whole insect. A following + denotes inclusion of pheromone emitting structures in the sample, a – the opposite.

Species	Origin	Body parts	Major tetrahydrofurans
Arctiidae			
Creatonotos transiens	Indonesia	BO–	not detected
Panaxia quadripunctaria	Germany	BO–	not detected
Noctuidae			
Actebia fennica	Canada	BO+	not detected
Autographa gamma	Germany	BO+	not detected
Mamestra configurata	Canada	BO+	11/16
Pseudaletia (or *Mythimna*) *unipuncta*	Canada	BO+	not detected[1]
Spodoptera frugiperda	USA	LE+, CL+	13/16
Plutellidae			
Plutella xylostella	Canada	BO+	not detected
Pyralidae			
Homeosoma electellum	Canada	BO+	not detected
Ostrinia nubilalis	Canada	BO+	not detected
Tortricidae			
Choristoneura fumiferana	Canada	BO+	not detected
Choristoneura rosaceana	Canada	BO+	not detected

[1] Larvae of the 3rd instar contained 11/20 and 13/18 as major THFs, while 6th instar larvae did not contain any THFs.

The hydroxylated tetrahydrofurans always contain the hydroxy group at C-1 of the longer side chain. These alcohols, normally present only in small amounts, were identified by us in six ithomiine species: *Ceratinia tutia, Heterosais nephele, Hypomenitis andromica, Mechanitis lysimnia, M. polymnia,* and *Scada kusa.* In the last named species they make up the largest part of the THFs found in the cuticular lipids. As with the parent THFs, two peaks with similar mass spectra could be observed by separation on apolar gas chromatographic phases. They represent the *cis-* and *trans-*

compounds, because the *erythro-* and *threo cis*-compounds formed during our synthesis could not be separated by GC. Thus, which of the four possible diastereomers occur naturally is unknown.

The relative amount of THFs in the cuticular lipids varies from less than 5% in most species to more than 70% of total cuticular lipids in other species. As an example, the results of the analysis of the wing lipids of the ithomiine butterfly *Melinaea ludovica* are presented in Table III and Fig. 4. The THFs are the prominent lipids, especially 7/20-THF and 9/20-THF. They are accompanied by some hydrocarbons, aldehydes and steroids. Because small amounts of hydroxyl containing compounds did not show distinct peaks on our column, silylated extracts were analyzed. Thus, several hydroxy-compounds could be identified, including long chain secondary alcohols and hydroxylated THFs.

Table III. Constituents of the cuticular lipids of wings from *Melinaea ludovica* (Ithomiinae). Numbers (No) refer to Fig. 4. Relative concentrations are given (conc): +++ major component, ++ minor component, + trace component.

No	Compound	Conc
1	heptacosane	+
2	squalene	+
3	nonacosane	+
4	7/18-THF	+
5	triacontane	+
6	7/19-THF	+
7	6/20-THF	+
8	hentriacontane	+
9	cholesterol	++
10	9/18-THF	+
11	7/20-THF	+++
12	ergostenol	+
13	triacontanal	+
14	9/19-THF	++
15	8/20-THF	+
16	7/21-THF	+
17	stigmasterol	+
18	11/18-THF	+
19	9/20-THF	+++
20	10/20-THF	+
21	9/21-THF	+
22	13,21-dimethylpentatriacontane	++
23	docosanoic acid	+
24	tetracosanoic acid	+
25	hexacosanoic acid	+
26	8-nonacosanol	+
27	9-nonacosanol	+
28	10-nonacosanol	+
29	cholestadienol	+
30	9-triacontanol	+
31	7/1HO19-THF	+
32	11-hentriacontanol	+
33	7/1HO20-THF	++
34	13-tritriacontanol	+
35	9/1HO20THF	+

There is a very marked difference in the presence of THFs in the cuticular lipids of those butterflies and moths examined. For example, of the 60 species of butterflies investigated 87% had THFs while they were only found in 17% (two) of the 12 moth species examined. It should be noted that in the case of the butterflies, the choice of species and of the body parts examined was not systematic but rather associated with our interest in male sex pheromones. Thus the reported absence of THFs in certain species must be viewed with some caution. However, the moths were analysed specifically for the presence of THFs. Thus, in the species reported here to lack THFs, these lipids are truly absent from the cuticle rather than undetected due to a bias in sampling. It is clear that the relative frequency and role of THFs in different parts of the cuticles of adult butterflies and moths needs to be examined further.

Our studies show that THF levels in different development stages of a given species may also change during the insect's life cycle. While THFs are present in 3rd instar larvae (major components are 11/18-THF and 13/16-THF) of *P. unipuncta,* they were absent in both 6th instar larvae and adult males. This suggests that THFs may play a specific role at certain times in the insect's development.

Discussion

The results of our analysis show that the cuticular lipids of many Lepidoptera contain 2,5-dialkyltetrahydrofurans. In all cases in which different body parts were investigated, similar THF patterns were found. They are not restricted to a distinct body part. The species investigated were selected because of our interest in male butterfly pheromones, and not for complete coverage of the Lepidoptera. Therefore the conclusion that the THFs occur preferentially in Nymphalidae subfamilies should not be drawn, because the species selection is biased towards that family. Moreover, skipper butterflies (Hesperioidea) and moth groups now thought to be more closely related to the butterflies (e. g. Hedylidae, Uraniidae, Geo-

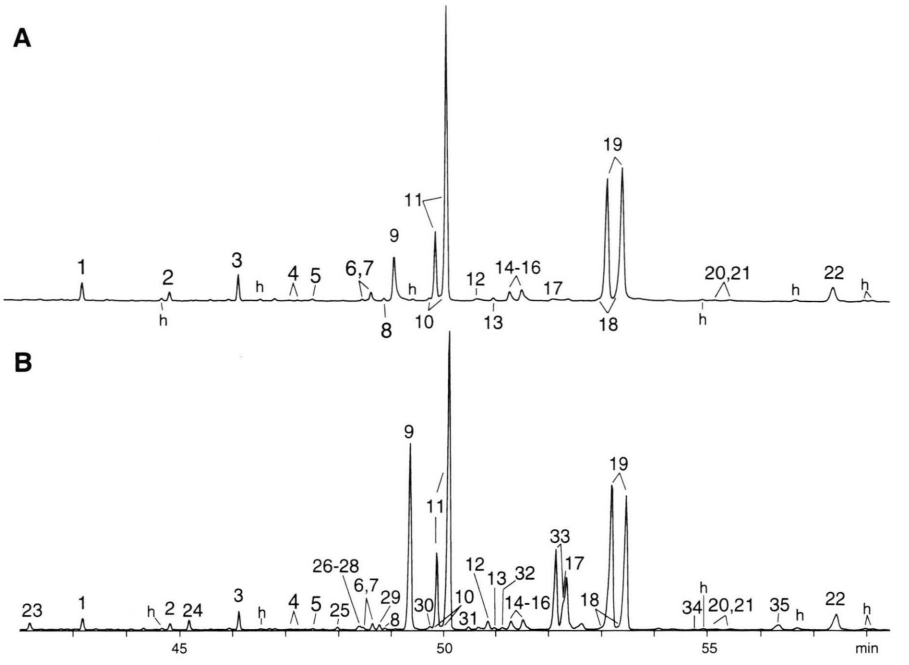

Fig. 4. Gas chromatogram of the wing cuticular lipids of *Melinaea ludovica.* Numbers refer to Table III. A: extract of cuticular lipids; B: extract after silylation; numbers of compounds containing hydroxyl groups refer to the respective silylated products. h: hydrocarbon.

metridae: de Jong *et al.* (1996)) have not been sampled. Instead, we propose that the THFs constitute important components of cuticular lipids present in many Lepidoptera and other insect orders. They have also been identified in the locust *Schistocerca gregaria* (W. Francke, personal communication). Nevertheless, they seem to be more abundant in butterflies than in moths because we encountered moth species lacking them more often than such butterfly species.

Their distinctive mass spectra should ease the identification of further species containing THF. The simple 2,5-dialkyltetrahydrofurans described have not been reported from any natural source other than Lepidoptera before. They represent simple models of the acetogenic tetrahydrofurans identified from the plant family Annonaceae, which exhibit numerous pharmacological effects (Cavé *et al.,* 1996). Other ethers have only rarely been identified in insects so far (Nelson and Blomquist, 1995).

The biosynthesis of THFs is unknown. Interestingly, the major THFs of *Melinaea ludovica* are 7/20-THF and 9/20-THF. Minor components of the wing lipids were identified as 11-hentriacontanol and 13-tritriacontanol (see Table III). It is well known that secondary alcohols can cyclize preferentially to THFs under radical-forming conditions, giving a mixture of the respective *cis*- and *trans*-compounds (Mihailovic *et al.,* 1973). By radical cyclization of for example 11-hentriacontanol to the shorter side of the carbon chain, as depicted in Fig. 5, a mixture of *cis*- and *trans*-7/20-THF is obtained. This cyclization may be enzyme controlled, because the reaction takes place on one side only. Another possible biosynthetic precursor, the short-chain tetrahydrofurfuryl-ring-containing acid 5-(5-(1-hydroxyheptyl)-tetrahydrofurfur-2-yl)-pentanoic acid, was identified from wool fat (Ito *et al.,* 1971). Such tetrahydrofurfuryl acids (probably formed by oxidations of common unsaturated C_{16}- or C_{18} fatty acids) could be elongated by acetate units to the required chain length in a elongation-decarboxylation process similar to the biosynthesis of hydrocarbons (Nelson and Blomquist, 1995). Nevertheless, the varying chain length on both sides of the ring excludes one single acid as precursor. For the α-hydroxylated tetrahydrofu-

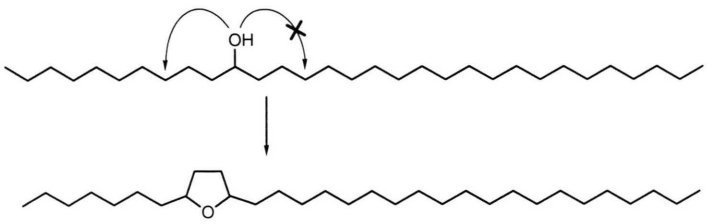

Fig. 5. Possible formation of 7/20-THF from 11-hentriacontanol by radical cyclization.

rans from the *Annonaceae* a biosynthetic pathway starting from 1,4-dienes has been proposed (Cavé *et al.*, 1996). After epoxidation of the double bonds, a ring opening-ring closure process can lead to such compounds. This model cannot explain the formation of the unfunctionalized THFs of the butterflies and it also does not explains their characteristic *cis/trans*-mixtures.

The primary function of the THF can be assumed to be to prevent desiccation of the insect, as has been established for other insect waxes (Nelson and Blomquist, 1995). However, it is well known that ethers can stabilize radicals at the α-position and are thus prone to autooxidation. The long side chains may further stabilize such radicals. Whether this leads to antioxidative properties of the THFs or in contrast promotes oxidative degradation of the wax layer or the cuticle remains unclear. Thus, given the marked differences observed between the occurrence of THFs in diurnal and nocturnal Lepidoptera, as well as within the life cycle of a single species, it is evident that considerably more research is required to elucidate their biosynthesis and function in insects with different life histories. Furthermore, from a broader chemical ecology perspective, the intraspecific differences may provide a better understanding of host-parasitoid interactions. For example, the majority of hymenopterous parasitoids attacking *P. unipuncta* larvae preferentially oviposit in early instar individuals. Thus, the presence of THFs in 3rd but not 6th instar larvae may play an important role in host selection if, as reported for the *P. separata-A. kariyai* system (Takabayashi and Takahashi, 1986a, b), these cuticular lipids serve as a kairomone for the parasitoids.

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